Left or right? Directions to stem cell engraftment

In this issue of JEM, Wu et al. (https://doi.org/10.1084/jem.20171341) use genetic barcoding of macaque hematopoietic stem cells to demonstrate that, after transplantation, HSCs are very asymmetrically distributed and uncover a thymus-independent pathway for mature T cell production in the bone marrow.

Insight from Mirjam E. Belderbos, Leonid Bystrykh, and Gerald de Haan

Hematopoietic stem cells (HSCs) produce all blood cell lineages for the entire lifespan of an organism. Therapeutically, transplantation of HSCs is used to treat patients with a variety of hematological disorders or metabolic diseases. HSC transplantation (HSCT) is arguably the only stem cell therapy that is routinely performed, now in hundreds of thousands of patients. Surprisingly, though, our understanding of the locational fate of transplanted HSCs is very limited. For instance, we do not know where HSCs land (or home) in the bone marrow, when and where HSC mobilization takes place, nor whether there are lineage-specific production sites in the marrow.

To address some of these questions, in this issue, Wu et al. studied the fate of peripheral blood $CD34^+$ hematopoietic stem and progenitor cells (HSPCs) and their progeny upon autologous transplantation in rhesus macaques. Using a genetic barcoding strategy, analogously as has been done previously in murine (Gerrits et al., 2010; Naik et al., 2013) and human xenotransplantation studies (Cheung et al., 2013; Brugman et al., 2015), the authors quantitatively tracked hundreds of HSPCs over time and space. At multiple time points after transplantation, barcode composition, representing the quantitative output of individual HSCs, was measured in peripheral blood cells, lymph nodes, and two anatomically distinct bone marrow sites (the left and right iliac crest). These analyses revealed that the transplanted HSC clones were highly asymmetrically localized across different anatomical sites. Despite rapid normalization of blood counts and bone marrow cellularity, HSC equilibration between different marrow sites took months to sometimes years. Notably, HSC mobilization followed a tri-phasic pattern, characterized by HSC homing, followed by local blood cell production at the site of (potential) engraftment, and only later mobilization and equilibration in distant sites. In addition, by comparing clonal patterns of more differentiated cells across different anatomical locations, the authors were able to delineate clonal hierarchies and preferred sites of production of certain cell types. For instance, they demonstrate that the clonal origins of $CD16^+$ and $CD56^+$ NK cells are distinct and that $CD16^-CD56^+$ NK cells are preferentially produced in the bone marrow, whereas $CD16^+CD56^-$ NK cell production preferentially takes place in extramedullary sites. Finally, the authors also identify the local, site-specific presence of $CD3^+$ T cell populations in bone marrow, which are clonally related to their surrounding $CD34^+$ HSCs, suggestive of their local, thymus-independent production.

These results, in conjunction with previous studies, demonstrate the value of genetic barcoding to assess HSC dynamics, revealing intriguing and hitherto unappreciated spatial asymmetry of normal HSCs after transplantation (Verovskaya et al., 2014; Bystrykh and Belderbos, 2016). Simultaneously, these findings raise multiple questions.

For instance, the mechanisms guiding HSC migration to and from specific anatomical sites remain unclear. Understanding these mechanisms is of great interest, as this may yield new therapeutic opportunities to enhance/accelerate HSC engraftment upon transplantation. The work by Wu et al. (2018) is consistent with a tri-phasic model of HSC engraftment and mobilization upon HSCT (see figure, part A). First, transplanted HSCs home and engraft in different anatomical sites, which is characterized by marked asymmetry. Although it is tempting to speculate that this early asymmetry reflects functional differences between HSCs and their niche, one should bear in mind that the observed clonal distribution patterns may also be explained by the single-cell nature of the barcoding technique. After all, a single HSC can only home and engraft to one anatomical site. As HSC niches in the left and right iliac crest are presumably highly similar, the initial differences in clonal composition between these locations observed by Wu et al. (2018) are likely stochastic. Nonetheless, in other studies, many differences have been identified in the composition of HSC niches between more distant anatomical sites, which have been postulated to drive HSC diversity (Morrison and Scadden, 2014). Accordingly, bidirectional communication between HSCs and their niche may dictate HSC homing and/or fate upon (stochastic) engraftment in a certain site.

The second phase of engraftment is characterized by local HSC proliferation, resulting in simultaneous detection of $CD34^+$ HSCs and their clonal progeny at the site of engraftment, but not at distant sites (see figure, part B). Although the clonal progeny of some

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HSCs can egress from their niche, the majority of HSC clones are still confined to their site of initial engraftment, resulting in marked asymmetry at the bone marrow level. Importantly, in the paper by Wu et al. (2018), the ability of HSCs to contribute to blood formation early after transplantation seemed to be independent of their clone size in the bone marrow, suggesting that factors other than proliferation allow for their mobilization. In clinical transplantation protocols, the interval between HSCT and blood cell recovery is the major predictor of patient outcome. Accordingly, insight into the mechanisms inducing HSC mobilization to blood may identify novel targets to accelerate engraftment and improve the outcome of clinical HSCT recipients.

Finally, the third phase of HSC engraftment consists of gradual equilibration over the skeleton over a period of months to years (see figure, part C), ultimately resulting in HSC symmetry, in which the majority of (large) HSC clones are present in all locations. Of note, as Wu et al. (2018) restricted their analysis to major clones, the presence of residual asymmetry of minor clones cannot be excluded.

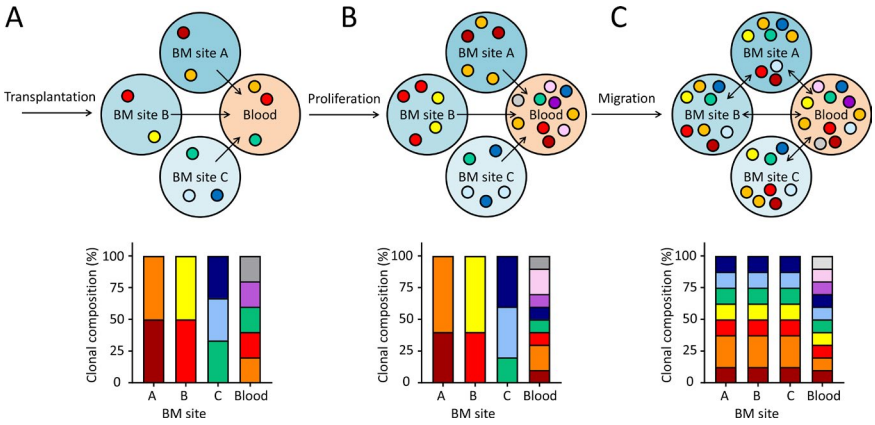
Regardless of the underlying mechanism, the findings by Wu et al. (2018) have important implications for fundamental studies on HSC biology, as well as for clinical HSCT procedures.

First, locational asymmetry may have a profound impact on the quantification of HSC frequency. Currently, most experimental strategies to quantify HSC frequency rely on (xeno)-transplantation of a population of hematopoietic stem/progenitor cells, either labeled or in limiting dilution (Bystrykh et al., 2012; Cheung et al., 2013). Subsequently, HSC frequency is calculated by dividing the number of retrieved clones by the administered cell dose. However, if measurements are performed in a single location, before HSCs have fully equilibrated, this may result in a large number of clones remaining undetected and an underestimation of true HSC frequency.

Second, locational asymmetry affects our understanding of clonal hierarchy. In the study by Wu et al. (2018), the relative abundance of individual clones varied according to the location sampled. Especially at early time points after transplantation, the majority of dominant clones in the left iliac crest were minor in the right iliac crest, and vice versa. Accordingly, clonal abundance in a single location may not necessarily reflect dominance in the total body (i.e., if all sites were analyzed). In fact, clonal dominance in the peripheral blood (referred to as clonal hematopoiesis of indeterminate potential), which has now been well established to occur during ageing in humans (Steensma et al., 2015), may not reflect clonal dominance at the bone marrow stem cell level. In addition, a decreasing size of a specific clone in one location may not reflect its decreased fitness compared with other clones, but may also indicate enhanced capacity to mobilize and engraft in other sites. Altogether, elaborate clone-tracking strategies, sampling multiple skeletal sites at multiple time points, will be needed to fully assess and discriminate HSC clonal complexity and dynamics.

Third, because of the lack of unique markers to identify HSCs and their clonal offspring in humans, their anatomical distribution in human recipients is still unknown. Nonetheless, the study by Wu et al. (2018) implies that sampling of a single skeletal site early after transplantation may not be sufficient to monitor engraftment and that clonal analyses in blood may be more representative. In the future, single-cell next generation sequencing technology may allow investigation of HSC dynamics and locational distribution in human recipients. These studies will be essential to determine how interactions between the bone marrow niche and transplanted donor HSC influence HSC fate, how factors like donor source and conditioning regimen impact HSC distribution, and how these can be exploited to enhance bone marrow reconstitution upon HSCT.

Finally, similar to the spatial asymmetry of normal HSCs observed in the current study, spatial diversity has also been shown to occur in malignancy. In solid tumors, the spectrum of genetic mutations differs between different locations within an individual tumor (Eirew et al., 2015; Lan et al., 2017) and between the primary tumor and metastatic sites (Turajlic and Swanton, 2016).



A tri-phasic model of HSC engraftment. The top circle graphs demonstrate HSC distribution across different anatomical locations in several phases after transplantation. The bottom bar graphs show the resulting clonal complexity. (A) Early after transplantation, HSC distribution is characterized by marked asymmetry intrinsic to single-cell engraftment. (B) Subsequently, HSC proliferation produces clonal offspring, which are initially largely confined to their initial site of production. Only few clones (for as yet unknown reasons) have the capacity to mobilize, which is clone-size independent. (C) Only months to years after transplant, HSC composition equilibrates across different sites. In the example given, at each time point, several clones is found in blood that is not present in any of the sampled bone marrow sites, suggestive of their production in other, nonsampled locations.

In recent xenograft studies, a similar process is suggested to apply to hematologic malignancies as well (Belderbos et al., 2017; Elder et al., 2017). This is of vital importance, as it suggests that clones may "hide" in certain skeletal locations and that sampling of a single bone marrow site, as is now common clinical practice, may be insufficient to capture full tumor heterogeneity. Moreover, as the local microenvironment can influence tumor cell properties, locational asymmetry may contribute to tumor heterogeneity and drive therapeutic resistance and disease relapse.

In sum, dissecting the degree of spatial asymmetry of normal HSCs and of their malignant counterparts will be of significant interest for our understanding of normal hematopoiesis and leukemia. In particular, it will be key to determine whether anatomical localization impacts on essential cell properties like proliferation, differentiation and sensitivity to therapeutic agents.

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